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The relationship of HLA-G 14-bp insertion/ deletion genetic polymorphism to the risk of multiple sclerosis and its clinical phenotypes

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Abstract

Background: Human leukocyte antigen (HLA)-G molecule has been suggested to have a potential immunomodulatory role in multiple sclerosis (MS). Genetic variant sites of HLA-G molecule have been reported to be associated with autoimmune diseases. Identifying the genetic risk factors of MS may help in preventive strategies and anticipating disease progression. The aim of this work was to investigate the effect of HLA-G 14-base-pair insertion/deletion (14-bp INS/DEL) genetic polymorphism on MS risk and clinical characteristics and to observe the clinical characteristics of the MS patients' group. The study included 48 MS patients and 50 cross-matched healthy controls, who were recruited from Sohag and Assiut university hospitals. Genetic testing (14-bp Ins/Del gene polymorphism) using polymerase chain reaction (PCR) was performed for patients and control groups. All patients had a detailed clinical assessment and have undergone measurement of disability using the Expanded Disability Status Scale (EDSS).

Results: No statistically significant difference was found between MS patients and healthy controls (HC) in genotypic and allelic frequencies of HLA-G 14-bp INS/DEL polymorphism (P=0.305). No significant association was found between HLA-G 14-bp INS/DEL polymorphism genotypes and clinical characteristics or degree of disability of MS patients. The most frequent presenting symptoms of MS were motor symptoms. Fatigue was the most reported symptom along the course of MS disease.

Conclusion: Although it has been long known that HLA-G represents an important MS susceptibility locus, in this study, no significant relation could be detected between the 14-bp INS/DEL polymorphism genotype and MS susceptibility. MS risk susceptibility may be not linked to a single allele but may depend on the combination of different polymorphic genetic sites. In this study, the lack of genetic susceptibility may be attributed to ethnic factor.

Keywords: HLA-G, Multiple sclerosis, Polymorphism, 14-bp insertion/deletion

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Background

Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS) with multifactorial interacting environmental and genetic factors [1]. The Middle East and North Africa are present in a moderate risk zone for MS based on the 2013 MS Atlas [2].

Whole genome studies concluded that the genetic site of human leucocyte antigen-G (HLA-G) represents an important MS susceptibility locus [3]. HLA-G is a nonclassical major histocompatibility complex (MHC) molecule where it is present at chromosome 6. It has important immunomodulatory and tolerogenic functions [4].

MHC genetic site plays a basic role in MS risk through the rs4959039 single nucleotide polymorphism (SNP) in the 3′ untranslated region (3′UTR) of the HLA-G gene [5].

Variable genetic polymorphisms at the 3′UTR site have been proposed to be involved including insertion/deletion (INS/DEL) of 14 base pairs (14 bp) polymorphism (rs1704) [6]. The 14-bp insertion/deletion (INS/DEL) polymorphism (rs66554220) present at the position +2960 in the exon 8 of the 3′UTR [7]. 14-bp insertion (INS) presence affects messenger ribonucleic acid (mRNA) splicing which contributes to lowering in HLA-G protein output, whereas the deletion allele (DEL) stabilizes the mRNA with higher HLA-G expression [8].

MS has markedly variable clinical characteristics and prognosis and some have early severe disability but others are still ambulatory and functioning for many years [9].

The aim of the current study is to investigate the relationship of genetic polymorphism of HLA-G 14-bp ins/del with MS risk and clinical characteristics in Egyptian MS patients.

Methods

Subjects

This case–control study included 52 patients who were recruited from inpatient neurology departments and outpatient neurology clinics and 50 healthy controls in a multicenter study. Four patients were excluded from the study. They were proved to have alternative diagnosis as systemic lupus erythematosus (one patient), Behcet disease (one patient), and other neurologic disorders (two patients with transverse myelitis).

Inclusion criteria

Forty-eight MS patients diagnosed with clinically definite MS (CDMS) were selected based on Revised McDonald's criteria (2017) [10]. Fifty age and sex cross-matched healthy individuals with no past medical history of autoimmune diseases or other neurologic diseases were included as the control group.

Ethical consideration

The study protocol was approved by the Ethics committee of the Faculty of Medicine in November 2016. Written informed consent was obtained from all participants after being informed that the confidentiality of their results will be kept along the whole study, and thereafter.

Methodology

All patients were subjected to complete clinical and neurologic assessment. CSF oligoclonal bands ($n=9$) and autoimmune profile ($n=15$) (included anti-double-stranded DNA, antiphospholipid antibodies, and ANA profile) were done when needed to confirm the diagnosis of MS and exclude other autoimmune diseases. Expanded Disability Status Scale (EDSS) [11] was used to assess neurological disability among patients. Patients were categorized according to the severity of their disease into mild (EDSS 0–3), moderate (EDSS 3.5–5.5), and severe (EDSS ≥6) [12].

14-bp Del/Ins gene polymorphism genetic testing

Patients and control groups were subjected to genetic assessment (14-bp Ins/Del gene polymorphism). Genomic DNA was brought out from blood by the method of salting. 14-bp Ins/Del genotyping was done by polymerase chain reaction (PCR) as described [13]. DNA was expanded and amplified with a set of primers: 5′-GTG ATG GGC TGT TTA AAG TGT CAC C-3′, 5′-GGA AGG AAT GCA GTT CAG CAT GA-3′. The 35 cycles of PCR were done at 94°C for 30 s, 64°C for 60 s, and 72°C for 60 s and final cycle of 72°C for 10 min. Electrophoresis of DNA segments was on 3% agarose gels containing ethidium bromide. The insertion allele was seen as a 127-bp band, while the deletion allele was visualized as 114-bp bands. The PCR was done as follows: 200 ng of genomic DNA was added to a final volume of 25 μl, with final concentrations as follows: PCR buffer 1×, 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 1.0 U of *Taq*-polymerase, and 10 pmol of each primer. Statistical analysis was performed by SPSS 18.0 software (SPSS, Inc., Chicago, IL). Quantitative data was represented as mean, standard deviation. The normality of the variables was checked using the Kolmogorov–Smirnov test. Accordingly, nonparametric tests (Mann–Whitney *U* test) were used to compare mean between the two categorical groups and the Kruskal–Wallis test was used to compare mean between the three or more groups when datasets were found to be not normally distributed. Correlations were assessed by the Spearman rank correlation coefficient. Statistical significance was assumed at the *P* value that was less than 0.05.

Results

The clinical manifestations of the initial presentation of MS patients are illustrated in Table 1. The clinical

Table 1 Clinical manifestations of initial presentation of MS patients. *n*, %

	Number	Percentage
Motor manifestations		
Hemiparesis	15	31.2%
Monoparesis	2	4.2%
Paraparesis	5	10.4%
Quadriparesis	1	2.1%
Cerebellar ataxia	8	16.7%
Total	31	64.6%
Visual manifestations		
Optic neuritis	10	20.8%
Sensory manifestations		
Superficial sensory impairment	1	2.1%
Deep sensory impairment	3	6.2%
Total	4	8.3%
Brain stem symptoms		
Trigeminal neuralgia	1	2.1%
Diplopia (INO)	2	4.2%
Total	3	6.3%
Multiple symptoms at onset		
Optic neuritis with weakness	5	10.4%
Diplopia (INO) with weakness	1	2.1%
Total*	6	12.5%
Recovery after first insult		
Complete	14	29.2%
Incomplete	31	64.6%
No recovery	3	6.3%

INO internuclear ophthalmoplegia. *12.5% (*n*=6/48) of patients presented with more than one symptom at onset: five patients presented with optic neuritis and hemiparesis and one patient presented with diplopia due to internuclear ophthalmoplegia and monoparesis

Table 2 Clinical manifestations of MS patients along MS course

	Number	Percentage
Ocular manifestations		
Optic neuritis		
Diminution of vision	19	39%
Field defects	12	25%
Abnormal color vision	2	4.1%
Total	33	68.1%
Ocular motor		
Nystagmus	11	22.9%
Internuclear ophthalmoplegia	7	14.5%
Ocular flutter	3	6.25%
Abducens palsy	2	4.16%
Total	23	47.9%
Motor manifestations		
Hemiparesis	20	41.7%
Monoparesis	4	8.3%
Paraparesis	5	10.4%
Quadriparesis	5	10.4%
Ataxia	12	25%
Total	46	95.8%
Other cranial nerve manifestations		
Trigeminal neuralgia	3	6.25%
Isolated facial upper motor	2	4.16%
Pseudoulbar palsy	1	2.1%
Total	6	12.5%
Sensory manifestations		
Superficial sensory impairment	21	43.8%
Deep sensory impairment	22	45.8%
Total	43	89.6%
Neurogenic bladder manifestations		
Precipitancy, urgency	19	39.6%
Incontinence	4	8.3%
Total	23	47.9%
Fatigue	38	79.2%
Spasticity	8	16.7%
Tonic spasms	22	45.8%
Ambulation		
Ambulant	32	66.7%
Assisted walk	10	20.8%
Wheelchair bound	6	12.5%

manifestations of MS patients along MS course are illustrated in Table 2.

There was no statistically significant difference in the genotypic and allelic frequencies of HLA-G 14-bp INS/DEL polymorphism between MS patients and controls. INS/INS was the most frequently encountered genotype in both MS patients and controls (62.5% and 70%, respectively) and the difference was statistically insignificant. The alleles having 14-bp insertion had higher frequencies (79% and 85% frequency for MS patients and controls, respectively) than deletion alleles (20.8% in patients and 15% in controls), and the difference was statistically insignificant (Table 3). There was no statistically significant relation between14-bp insertion/deletion polymorphism genotype and either different MS clinical phenotypes or disability scores (Table 4).

Discussion

The clinical presentation of MS has a wide range of variability which makes it more difficult to reach a confident clinical diagnosis. In the current study, as regards the clinical presentation of MS, the most frequent

Table 3 Comparison of allelic and genotypic frequencies of 14-bp insertion/deletion polymorphism of HLA-G gene between MS patients and control groups

	Case (*n*=48)	Control (*n*=50)	*P* value
Genotype			
14-bp INS/INS	30/48 (62.5%)	35/50 (70%)	0.305
14-bp INS/DEL	16/48 (33.3%)	15/50 (30%)	
14-bp DEL/DEL	2/48 (4.2%)	0/50 (0.0%)	
Allele			
14-bp INS	76 (79.2%)	85 (85%)	0.283
14-bp DEL	20 (20.8%)	15 (15%)	

14-bp INS/DEL 14-base pair insertion/deletion, *(%)* percentage of genotype in each group

presenting symptoms overall were motor symptoms (64.6%) (Table 1). This was consistent with studies carried out in KSA (61.8%) [14], Jordan (30.8%) [15], and Dubai (72.8%) [16] where motor symptoms were the most frequently encountered presenting symptoms of MS patients. In partial agreement with our findings in Iran, it was found that motor manifestations followed by sensory and then visual symptoms was the order of frequently presenting MS manifestations [17]. On the other hand, sensory impairment was the most frequent presenting symptom in Kuwait (49.2%) and Qatar (33.3%) [18, 19]. On the contrary, an earlier study in India found that visual symptoms (58%) were more frequent than motor symptoms among Indian MS patients [20].

The proportion of patients with no (6.3%) or incomplete (64.6%) recovery from the first insult (Table 1) was higher than that reported in Europe among French patients (17%) [21]. Higher levels of no or incomplete recovery after the first relapse among our patients may be attributed to the lack of awareness of MS symptoms and consequent delay in diagnosis and delay in treatment.

Fatigue is one of the main causes of impaired quality of life among MS patients [22]. Fatigue is also among the most common symptoms, reported by at least 75% of MS patients at some point in the disease course [23]. Similarly, 79.2% of patients in the present study reported a subjective sense of fatigue along the course of disease (Table 2). Although bladder manifestations are one of the most well-known symptoms of MS, with more than 90% of patients with MS experiencing bladder symptoms 10 years after disease onset [24], in the current study, only 47.9% of the sample had bladder symptoms (Table 2). This low rate might be explained by the variable duration of illness among MS patients (the mean duration of MS in our study was 5.2±4.8 years with a range 0.5–19 years) and low rate of spinal cord involvement in our sample (10/48=20.8%) together with the partial nature of spinal cord MS pathology.

It has been reported that HLA-G polymorphism has a potential contribution to MS susceptibility [25]. Group of studies have investigated the possible relation between variable HLA-G genetic polymorphisms and MS [4, 5, 26]. HLA-G expression in MS patients is enhanced on CSF macrophages, monocytes, microglia, and endothelial cells at sites of demyelination [27]. It was reported that

Table 4 Relation of 14-bp insertion/deletion polymorphism genotype to clinical, laboratory, cognitive, and psychological assessment of MS patients

	Genotype			*P*-value
	14-bp INS/INS (*n*=30)	14-bp INS/DEL (*n*=16)	14-bp DEL/DEL (*n*=2)	
Patients' age at presentation				
Mean±SD	32.3±8.7	30.6±5.9	26.5±12.0	0.601
Range	16–49	20–41	18–35	
Age at MS onset years				
Mean±SD	26.5±7.7	25.9±6.1	24.0±11.3	0.412
Range	14–45	18–36	16–32	
MS duration				
Mean±SD	5.9±5.3	4.2±4.1	2.5±0.7	0.897
Range	0.5–19	0.5–14	2–3	
Total number attacks				
Mean±SD	3.3±2.5	3.7±2.9	2.5±0.7	0.861
Range	1–10	1–10	2–3	
EDSS score				
Mean±SD	4.3±2.3	3.9±1.7	3.0±0.0	0.372
Range	1–9	2–7	3–3	

the 14-bp DEL/ + 3142 G combined genotypic frequency was related significantly to MS risk [28]. To the best of our knowledge, there are no available data regarding this issue for Egyptian patients with MS.

The present study showed no significant difference between patients and controls regarding genotypic and allelic frequencies of 14-bp INS/DEL polymorphism reflecting an insignificant risk of this polymorphism to MS susceptibility (P=0.305) (Table 3). Similarly, a study from Tunisia demonstrated that there was a lack of association between the 14-bp INS/DEL polymorphism and the risk of having MS [28]. Moreover, Kroner and colleagues studied three polymorphic sites in the HLA-G genetic site including the 14-bp INS/DEL polymorphism and found no relation between any of these three polymorphisms and MS in the German population [4]. Furthermore, a meta-analysis was carried out to find the link between the 14-bp INS/DEL polymorphism and various autoimmune diseases. It concluded that this polymorphism is not linked to MS and rheumatoid arthritis, but associated with several other autoimmune diseases such as systemic lupus erythematosus and Crohn's disease. This finding suggests that this genetic polymorphism functionality is disease dependent. Thus, HLA-G 14-bp INS/DEL polymorphism is not a usual genetic factor for different autoimmune disorders but other various pathogenic mechanisms are linked to the emergence of polygenetic autoimmune disorders [29].

On the other hand, an Iranian study demonstrated that HLA-G 14-bp insertion/deletion polymorphism could be a risk factor for MS susceptibility [25].

The controversy between studies of HLA-G 14-bp INS/DEL polymorphism could be attributed to variance in sample sizes, the ethnic groups, and methods used in different studies besides variable environmental factors. This leads to a suggestion that other different HLA-G polymorphic sites may work together to affect HLA-G expression in MS.

However, Fredj and his colleagues found that 14-bp DEL/ + 3142 G haplotype combined genotype (+3142 G another studied polymorphic site) had significantly increased frequency in MS patients compared to controls [28].

Wisniewski and coauthors when evaluating the -725C > G > T, -716T > G, and 14-bp INS/DEL HLA-G polymorphisms in MS have found that the -725G/-716T/14-bp DEL haplotype may be a susceptibility factor to this disease in the Polish population [30]. This confirms that the MS risk susceptibility may be not linked to a single allele but may depend on the combination of different polymorphic genetic sites.

The current study found no association between the 14-bp INS/DEL polymorphism genotypes and clinical characteristics or disease disability of MS patients assessed by EDSS (Table 4). Similarly, Fredj and his

colleagues reported that the 14-bp INS/DEL polymorphism genotype had no effect on the initiation of the disease [28]. Furthermore, previous studies found no association between HLA-G 14-bp INS/DEL gene polymorphism and the severity of MS [4, 25, 28]. On the other hand, it has been reported that MS patients with 14-bp INS/INS and 14-bp INS/DEL genotypes are more early disease onset compared to patients with 14-bp DEL/DEL genotype [30]. However, in the current study, patients with 14-bp INS/INS and 14-bp INS/DEL genotypes tend to have a later age of MS onset (Table 4) but the difference was insignificant.

Taken together, the results of this study emphasize that the HLA-G 14-bp INS/DEL polymorphism alone does not affect MS susceptibility. This means that other polymorphic genetic sites may have an important role in the regulation of the risk of developing MS. Thus, it can be confirmed that different polymorphic sites interact to modulate the HLA-G role in MS. Therefore, a more extensive research is needed to better clarify the interactive relation between the 14-bp INS/DEL polymorphism, other HLA-G polymorphic sites, and MS. Moreover, further investigations are needed to evaluate detailed HLA-G polymorphisms and transcription and translation rate of the HLA-G gene under different pathological and normal conditions.

Conclusion

The HLA-G 14-bp INS/DEL polymorphism alone may be not a genetic risk factor for MS, but it may be involved together with other HLA-G polymorphic sites in different pathogenic mechanisms that related to the development of polygenic autoimmune diseases like MS.

Availability of data and materials
The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Ethics approval and consent to participate
The study protocol was approved by the Ethics committee of Sohag Faculty of Medicine in November 2016 (the committee reference number is not applicable). Written informed consent was obtained from all participants after being informed that the confidentiality of their results will be kept along the whole study, and thereafter.

Consent for publication
Written informed consent was obtained from all participants.

Competing interests
The authors declare that they have no competing interests.

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